

following amendments and consideration of the following remarks.

Amendments to the Description

Please delete the material from page 20, line 20, to page 21, line 16.

Please replace the paragraph commencing on page 21, line 17, with the following re-written paragraph.

-- Figure ~~6~~ 1 shows a protein sequence alignment of the members of the triacylglycerol lipase gene family (SEQ ID Nos: ~~13-15~~ 9-11). Shaded residues are identical to the LLGXL protein (SEQ ID NO: ~~8~~ 6). The polynucleotide sequence at the top is the coding portion of the nucleic acid encoding LLGXL protein (nucleotides 0 to 1751 of SEQ ID NO: ~~7~~ 5). The deduced amino acid sequence of human LIPG(EL; endothelial lipase) (SEQ ID NO: ~~8~~ 6) is provided on the top line and is compared with the other major members of the TG lipase family, LPL (lipoprotein lipase) (SEQ ID NO: ~~13~~ 9), HL (hepatic lipase) (SEQ ID NO: ~~14~~ 10) and PL (pancreatic lipase) (SEQ ID NO: ~~15~~ 11). EL residues identical to those in at least one other member of the family are shaded as well as the corresponding residue in the other family member. Amino acids are numbered according to convention beginning with the initial residue of the secreted protein. The predicted sites of signal peptide cleavage are marked with a solid line between amino acid residues. The GXSXG lipase motif containing the active serine is boxed. The amino acids of the catalytic triad are marked with an asterisk. The conserved cysteines are marked with filled circles. Potential N-linked glycosylation sites are marked with arrowheads. The lid region is indicated by a bold line. Gaps were introduced into the sequences to maximize the alignment values using the CLUSTAL program.--

Please replace the paragraph commencing on page 22, line 7, with the following re-written paragraph.

--Figure 7 2 shows a northern analysis of LIPG mRNA in THP-1 cells. Cells were stimulated with either PMA or PMA and oxidized LDL (PMA + oxLDL). Numbers at the left indicate the positions of RNA standards (in kilobases).--

Please replace the paragraph commencing on page 22, line 11, with the following re-written paragraph.

--Figure 8 3 shows a northern-blot analysis of expression of LIPG mRNA compared with LPL in human tissues. A blot containing mRNA from the indicated human tissues was incubated with radiolabelled LPL and  $\beta$ -actin (ACTB) probes as described.--

Please replace the paragraph commencing on page 22, line 16, with the following re-written paragraph.

--Figure 9 4 shows a Northern-blot analysis of cultured cell lines. The panel on the left (lanes 1-6) was hybridized with the LIPG(EL) probe and that on the right (lanes 7-12) with the LPL probe. Lanes 1, 7, unstimulated HUVEC; lanes 2, 8, HUVEC stimulated with PMA; lanes 3, 9, HUVEC stimulated with thrombin; lanes 4, 10, unstimulated HCAEC; lanes 5, 11, HCAEC stimulated with PMA; lanes 6, 12, THP-1 stimulated with PMA.--

Please replace the paragraph commencing at page 22, line 23, with the following re-written paragraph.

-- Figure 10 5 shows the sequence of the immunizing peptide (SEQ ID NO: ~~16~~ 12) and its relation to the LLGXL protein sequence (SEQ ID NO: 8 6). The peptide is shown

in the shaded box. The terminal cysteine was introduced to aid coupling of the peptide to the carrier protein.--

Please replace the paragraph commencing at page 22, line 28, with the following re-written paragraph.

--Figure ~~11~~ 6 shows the results obtained when conditioned media from HUVEC and HCAEC were subjected to immunoblot analysis with rabbit anti-EL peptide antiserum. Lane 1, unconditioned media; lane 2, unstimulated HUVEC; lane 3, HUVEC stimulated with PMA; lane 4, unstimulated HCAEC; lane 5, HCAEC stimulated with PMA.--

Please replace the paragraph commencing at page 23, line 5, with the following re-written paragraph.

--Figure ~~12~~ 7 shows a western analysis of heparin-Sepharose bound proteins in conditioned medium from COS-7 cells transiently transfected with an expression vector containing a cDNA for LLGN or LLGXL or no DNA (Mock). Proteins from PMA-stimulated endothelial cells (HCAEC + PMA) were included for size reference. Numbers to the left indicate the apparent molecular weight of the major immunoreactive proteins as determined by a comparison to protein standards.--

Please replace the paragraph commencing at page 23, line 13, with the following re-written paragraph.

-- Figure ~~13~~ 8 shows the sequence of the rabbit LIPG PCR product (RLLG.SEQ, SEQ ID NO: ~~11~~ 7) and the sequence alignment between the rabbit LIPG PCR product and the corresponding sequence in the human cDNA (LLG7742A) (nucleotides 1023 to 1247 of SEQ ID NO: ~~7~~ 5). Identical nucleotides are shaded.--

Please replace the paragraph commencing at page 23, line 18, with the following re-written paragraph.

--Figure ~~14~~ 9 shows the phospholipase A activity of human EL-AS, EL and LPL using a phosphatidylcholine substrate. To perform the assay 700μl of conditioned medium harvested from COS-7 cells transiently transfected with either pcDNA3.0/LIPG-AS, LIPG, or LPL expression constructs were assayed in triplicate for phospholipase activities as described below. Following a two hour incubation at 37°C, reactions were terminated, and 14C labeled free fatty-acid was extracted, and counted to determine the amount of free fatty-acid produced.--

Please replace the paragraph commencing at page 23, line 28, with the following re-written paragraph.

-- Figure ~~15~~ 10 shows the triacylglyceride lipase activity of human EL-AS, EL and LPL using a triolein substrate. To perform the assay 700μl of conditioned medium harvested from COS-7 cells transiently transfected with either pcDNA3.0/LIPG-AS, LIPG, or LPL expression constructs was assayed in triplicate for triglyceride activities described below. Following a two hour incubation at 37°C, reactions were terminated, and 14C labeled free fatty-acid was extracted, and counted to determine the amount of free fatty-acid produced.

Please replace the paragraph commencing at page 24, line 9, with the following re-written paragraph.

--Figure ~~16~~ 11 shows the hybridization of LIPG and LPL probes to genomic DNAs from different species.--

Please replace the paragraph commencing at page 24, line 11, with the following re-written paragraph.

-- Figure ~~17~~ 12 shows expression of LIPG in the liver of a wild-type mouse 5 days after AdhEL injection. Lane 1, liver from mouse injected with Adnull; lane 2, liver from mouse injected with AdhEL.--

Please replace the paragraph commencing at page 24, line 15, with the following re-written paragraph.

-- Figure ~~18~~ 13 shows plasma levels of HDL cholesterol in AdhEL- and Adnull-injected wild-type mice.--

Please replace the paragraph commencing at page 24, line 17, with the following re-written paragraph.

-- Figure ~~19~~ 14 shows lipoprotein profiles in wild-type mice injected with AdhEL and Adnull at baseline before injection (left) and 14 days after injection (right).--

Please replace the paragraph commencing at page 24, line 20, with the following re-written paragraph.

-- Figure ~~20~~ 15 shows HDL cholesterol levels in human apoA-I transgenic mice after injection with Adnull or AdhEL.--

Please replace the paragraph commencing at page 24, line 22, with the

following re-written paragraph.

-- Figure ~~21~~ 16 shows ApoA-I levels in human apoA-1 transgenic mice after injection with Adnull or AdhEL.--

Please replace the paragraph commencing at page 24, line 24, with the following re-written paragraph.

-- Figure ~~22~~ 17 shows the effect of injection of AdhEL in LDL receptor-deficient mice on VLDL/LDL cholesterol levels.--

Please replace the paragraph commencing at page 24, line 26, with the following re-written paragraph.

-- Figure ~~23~~ 18 shows the effect of AdhEL on HDL receptor-deficient mice on HDL cholesterol levels.--

Please replace the section entitled "Description of the Sequences", inserted previously at the end of page 24 by amendment on October 18, 2004, with the following re-written section.

--

DESCRIPTION OF THE SEQUENCES

SEQ ID NO. 1 is the nucleic acid sequence of the differential display ~~PT-PCR~~ RT-PCR product containing a portion of the cDNA encoding human LIPG polypeptide.

SEQ ID NO. 2 is the deduced amino acid sequence encoded by SEQ ID NO. 1.

SEQ ID NO. 3 is the nucleic acid sequence of the 5' RACE extension of the cDNA fragment of SEQ ID NO. 1.

SEQ ID NO. 4 is the deduced amino acid sequence encoded by SEQ ID NO. 3.

~~SEQ ID NO. 5 is the nucleic acid sequence of the cDNA encoding human LLGN polypeptide. This cDNA corresponds to an mRNA product formed from transcription of the human LIPG gene.~~

~~SEQ ID NO. 6 is the deduced amino acid sequence encoded by SEQ ID NO. 5 (the sequence for human LLGN polypeptide).~~

SEQ ID NO. 5 7 is the nucleic acid sequence of the cDNA encoding human LLGXL polypeptide. This cDNA corresponds to an mRNA product formed from transcription of the human LIPG gene.

SEQ ID NO. 6 8 is the deduced amino acid sequence encoded by SEQ ID NO. 5 7 (the sequence for human LLGXL polypeptide).

~~SEQ ID NO. 9 is the nucleic acid sequence encoding SEQ ID NO. 10.~~

~~SEQ ID NO. 10 is the amino acid sequence common to both SEQ ID NOS. 6 and 8.~~

SEQ ID NO. ~~11~~ 7 is the nucleic acid encoding the rabbit LIPG PCR product.

SEQ ID NO. ~~12~~ 8 is the deduced amino acid sequence encoded by SEQ ID NO. ~~11~~ 7.

SEQ ID NO. ~~13~~ 9 is the amino acid sequence for human lipoprotein lipase (LPL).

SEQ ID NO. ~~14~~ 10 is the amino acid sequence for human hepatic lipase (HL).

SEQ ID NO. ~~15~~ 11 is the amino acid sequence for human pancreatic lipase (PL).

SEQ ID NO. ~~16~~ 12 is the amino acid sequence of an immunizing peptide corresponding to residues 8 to 23 of LLGXL polypeptide.

SEQ ID NO. ~~17~~ 13 is the nucleic acid sequence for differential display downstream primer 7.

SEQ ID NO. ~~18~~ 14 is the nucleic acid sequence for differential display upstream primer 15.

SEQ ID NO. ~~19~~ 15 is the nucleic acid sequence for 5' RACE Primer 2a.



SEQ ID NO. ~~20~~ 16 is the nucleic acid sequence for 5' RACE Primer 3a.

SEQ ID NO. ~~24~~ 17 is the nucleic acid sequence for 5'RACE Primer 4a.

SEQ ID NO. ~~22~~ 18 is the nucleic acid sequence for 5' RACE anchor primer.

SEQ ID NO. ~~23~~ 19 is the nucleic acid sequence for the 5' RACE universal amplification primer.

SEQ ID NO. ~~24~~ 20 is the nucleic acid sequence for 5' LPL primer.

SEQ ID NO. ~~25~~ 21 is the nucleic acid sequence for 3' LPL primer.

SEQ ID NO. ~~26~~ 22 is the nucleic acid sequence for primer DLIP774.

SEQ ID NO. ~~27~~ 23 is the nucleic acid sequence for primer LLGgen2a.

SEQ ID NO. ~~28~~ 24 is the nucleic acid sequence for Hllg-gsp1 primer.

SEQ ID NO. ~~29~~ 25 is the nucleic acid sequence for Hllg-gsp2a primer.

SEQ ID NO. ~~30~~ 26 is the nucleic acid sequence for G3PDH 5' primer.

SEQ ID NO. ~~34~~ 27 is the nucleic acid sequence for G3PDH 3' primer. - -

Please delete the material added by amendment on November 6, 2002 at page 25, between lines 7 and 8.

Page 25, line 8, please add the following 2 paragraphs which reinstate matter from the application as filed.

**--The Enzymatic Activity of the LIPG Gene Product**

The present invention relates to methods for regulating the levels of HDL cholesterol and apolipoprotein AI, VLDL cholesterol and LDL cholesterol utilizing methods and compositions which lower or raise the activity of the LIPG lipase enzyme. In particular, the present invention is based in part on the discovery of the enzymatic activity of the polypeptide products of the LIPG gene on HDL cholesterol and apolipoprotein AI, VLDL cholesterol and LDL cholesterol. The polypeptide products of LIPG are members of the triacylglycerol lipase family and comprise an approximately 39 kD catalytic domain of the triacylglycerol lipase family. Because this newly discovered lipase was found to be synthesized by endothelial cells and this is a unique feature compared with other members of the triacylglycerol lipase family, this lipase has been named "endothelial lipase" (EL). Because the LIPG gene will be discussed extensively in the sections which follow, EL will be hereinafter referred to as LIPG polypeptide, for the purposes of clarity. In general, the LIPG polypeptide is found in two major forms, referred to hereinafter as "the LLGN polypeptide" and "the LLGXL polypeptide." The LLGN polypeptide, has 354 amino acids. The LLGXL polypeptide has 500 amino acids and exhibits 43% similarity to human lipoprotein lipase and 37% similarity to human hepatic lipase. As used herein, the term "LIPG polypeptide" or "LIPG protein" encompasses both LLGN and LLGXL.

The sequence of the LIPG polypeptide contains the characteristic GX SXG lipase motif, a conserved catalytic triad, a 19-residue lid region, conserved heparin and lipoprotein binding sites and 5 potential N-linked glycosylation sites. The region with the greatest sequence divergence in the triacylglycerol lipase family is the lid domain, which forms an amphipathic helix covering the catalytic pocket of the enzyme (Winkler et al., *Nature*, 343, 771-774 (1990); van Tilbeurgh et al., *J. Biol. Chem.*, 269, 4626-4633 (1994)) and confers substrate specificity to the enzymes of this family (Dugi et al., *J. Biol. Chem.*, 270, 25396-25401 (1995)). The 19-residue lid region of LIPG is three residues shorter and less amphipathic than those found in lipoprotein lipase and hepatic lipase, consistent with a different enzymatic profile. The predicted molecule weight of the mature protein is approximately 55 kD; a 68 kD form is likely to be a glycosylated form, whereas a 40 kD form may be the product of a specific proteolytic cleavage.--

Please delete the paragraph commencing on page 29, line 23.

Please replace the paragraph commencing on page 29, line 26, with the following re-written paragraph.

--"**LLGXL polypeptide**" and "**LLGXL protein**" mean a polypeptide including the sequence SEQ ID NO: 8 6, said polypeptide being glycosylated or non-glycosylated.--

Please replace the paragraph commencing on page 35, line 24, with the following re-written paragraph.

-- The present invention utilizes polypeptides encoded by LIPG which are members of the triacylglycerol lipase family, and which comprise a 39 kD catalytic domain of the triacylglycerol lipase family, ~~e.g., having the sequence SEQ ID NO: 10.~~  
~~In certain embodiments of the present invention, an isolated LIPG polypeptide comprising the sequence SEQ ID NO: 6 and having an apparent molecular weight of about 40 kD on a 10% SDS-PAGE gel is utilized.~~ In another an embodiment of the present invention, an isolated LIPG polypeptide comprising the sequence SEQ ID NO: 8 and having an apparent molecular weight of about 55 kD or 68 kD on a 10% SDS-PAGE gel is utilized. ~~In yet another embodiment, the polypeptides utilized in the present invention are subfragments of these polypeptides. In still yet another embodiment, the polypeptides used in the present invention are antibodies capable of binding to an LIPG polypeptide.--~~

Please replace the paragraph commencing on page 46, line 17, with the following re-written paragraph.

-- The antisense nucleic acids can also be DNA sequences whose expression in the cell produces RNA complementary to all or part of the LIPG mRNA. Antisense nucleic acids can be prepared by expression of all or part of a sequence selected from the group consisting of SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. ~~7~~ 5, or SEQ ID No. ~~11~~ 7, in the opposite orientation, as described in EP 140308. Any length of antisense sequence is suitable for practice of the invention so long as it is capable of down-regulating or blocking expression of LIPG. Preferably, the antisense sequence is at least 20 nucleotides in length. The preparation and use of antisense nucleic acids, DNA encoding antisense RNAs and the use of oligo and genetic antisense is disclosed in WO92/15680, the contents of which are incorporated herein by reference.--

Please replace the paragraph commencing on page 77, line 18, with the following re-written paragraph.

-- To reamplify the PCR product, 26.5 microliters of the eluted DNA were used in a amplification reaction that also included 5  $\mu$ l 10x PCR buffer, 3  $\mu$ l 25 mM  $MgCl_2$ , 5  $\mu$ l 500  $\mu$ M dNTPs, 5  $\mu$ l 2 $\mu$ M downstream primer 7, 7.5  $\mu$ l upstream primer 15, and 0.5  $\mu$ l Amplitaq polymerase. The PCR cycling parameters and instrument were as described above. Following amplification, 20  $\mu$ l of the reamplification was analyzed on an agarose gel and 4  $\mu$ l was used to subclone the PCR products into the vector pCRII using the TA cloning system (Frohman, M.A., Dush, M.K., and Martin, G.R. (1988) Proc. Natl. Acad. Sci. USA 85,8998-9002) (Invitrogen). Following an overnight ligation at 14°C, the ligation products were used to transform E. coli. Resulting transformants were picked and 3 ml overnight cultures were used in plasmid minipreparations. Insert sizes were determined using EcoRI digestions of the plasmids and clones containing inserts of the approximate size of the original PCR product were sequenced using fluorescent dye-terminator reagents (Prism, Applied Biosystems) and an Applied Biosystems 373 DNA sequencer. The sequence of the PCR product is SEQ ID NO. 1 ~~shown in Figure 2~~. The sequence of the amplification primers is underlined.--

Please replace the paragraph commencing on page 78, line 13, of the application with the following re-written paragraph.

--1 $\mu$ l (1  $\mu$ g) of RNA was combined with 3  $\mu$ l (3 pmol) primer 2a and 11  $\mu$ l DEPC-treated water and heated to 70°C for 10 minutes followed by 1 minute on ice. 2.5  $\mu$ l 10x reaction buffer (200 mM Tris-HCl pH 8.4, 500 mM KCl), 3  $\mu$ l 25 mM

MgCl<sub>2</sub>, 1 µl 10 mM dNTP mix, and 2.5 µl 0.1 M DTT were added. The mix was incubated at 42°C for 2 minutes, then 1 µl Superscript II reverse transcriptase was added. The reaction was incubated for an additional 30 minutes at 42°C, 15 minutes at 70°C, and on ice for 1 minute. One microliter of RNase H (2 units) was added and the mixture was incubated at 55°C for 10 minutes. The cDNA was purified using the GlassMax columns (Sambrook, J. Fritsch, E.F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, second edition, Cold Spring Harbor Laboratory Press, Plainview, NY) included in the kit. The cDNA was eluted from the column in 50 µl dH<sub>2</sub>O, lyophilized, and resuspended in 21 µl dH<sub>2</sub>O. Tailing of the cDNA was accomplished in the following reaction: 7.5 µl dH<sub>2</sub>O, 2.5 µl reaction buffer (200 mM Tris-HCl pH 8.4, 500 mM KCl), 1.5 µl 25 mM MgCl<sub>2</sub>, 2.5 µl 2 mM dCTP, and 10 µl of the cDNA were incubated at 94°C for 3 minutes, then 1 minute on ice. 1 µl (10 units) of terminal deoxynucleotidyl transferase was added and the mixture was incubated for 10 minutes at 37°C. The enzyme was heat inactivated by incubation at 70°C for 10 minutes and the mixture was placed on ice. PCR amplification of the cDNA was performed in the following steps: 5 µl of the tailed cDNA was included in a reaction which also contained 5 µl 10x PCR buffer (500 mM KCl, 100 mM Tris-HCl pH 8.3, 15 mM MgCl<sub>2</sub>, and 0.01% (w/v) gelatin), 1 µl 10 mM dNTP mix, 2 µl (10 pmol) anchor primer, 1 µl (20 pmol) primer 3a, and 35 µl dH<sub>2</sub>O. The reaction was heated to 95°C for 1 minute, then 0.9 µl (4.5 units) Amplitaq polymerase was added. The reaction was cycled 40 times under the following conditions: 94°C for 5 seconds, 50°C for 20 seconds, and 72°C for 30 seconds. One microliter of this reaction was used in a nested reamplification to increase levels of specific product for subsequent isolation. The reamplification included: 1 µl primary amplification, 5 µl 10x PCR buffer, 1 µl 10 mM dNTP mix, 2 µl (20 pmol) universal amplification primer, 2 µl (20 pmol) primer 4a, and 38 µl dH<sub>2</sub>O. The reaction was heated to 95°C

for 1 minute, then 0.7  $\mu$ l (3.5 units) Amplitaq polymerase was added. The reaction was cycled 40 times under these conditions; 94°C for 5 seconds, 50°C for 20 seconds, and 72°C for 30 seconds. The amplification products were analyzed via 0.8% agarose gel electrophoresis. A predominant product of approximately 1.2 kilobase pairs was detected. Two microliters of the reaction products were cloned into the pCRII vector from the TA cloning kit (Invitrogen) and incubated at 14°C overnight. The ligation products were used to transform *E. coli*. The insert sizes of the resulting transformants were determined following EcoRI digestion. Clones containing inserts of the approximate size of the PCR product were sequenced using fluorescent dye-terminator reagents (Prism, Applied Biosystems) and an Applied Biosystems 373 DNA sequencer. The sequence of the RACE product including the EcoRI sites from the TA vector is SEQ ID NO. 3 ~~are shown in Figure 3. The sequences of the amplimers (universal amplification primer and the complement to 5'RACE primer 4a) are underlined. --~~

Please replace the paragraph commencing on page 81, line 22, with the following re-written paragraph.

-- The library was probed using established methods (Walter, P., Gilmore, R., and Blobel, G. (1984) Cell 38,5-8). Briefly, the filters were hybridized for 24 hours at 65°C in 4.8X SSPE (20X SSPE = 3.6 M NaCl, 0.2 M NaH<sub>2</sub>PO<sub>4</sub>, 0.02 M EDTA, pH 7.7), 20 mM Tris-HCl pH 7.6, 1X Denhardt's solution (100X= 2% Ficoll 400, 2% polyvinylpyrrolidone, 2% BSA), 10% dextran sulfate, 0.1% SDS, 100  $\mu$ g/ml salmon sperm DNA, and 1x10<sup>6</sup> cpm/ml radiolabelled probe. Filters were then washed three times for 15 minutes at room temperature in 2X SSC (1X SSC = 150 mM NaCl, 15 mM sodium citrate pH 7.0), 0.1% sodium dodecyl sulfate (SDS) followed by three

washes for 15 minutes each at 65°C in 0.5X SSC, 0.1% SDS. Phage which hybridized to the probe were isolated and amplified. DNA was purified from the amplified phage using LambdaSorb reagent (Promega) according to the manufacturer's instructions. The inserts were excised from the phage DNA by digestion with EcoRI. The inserts were subcloned into the EcoRI site of a plasmid vector (Bluescript II SK, Stratagene). The sequence of the open reading frame contained within the 2.6 kb EcoRI fragment of the cDNA was determined by automated sequencing as described above. The sequence is SEQ ID NO. 5 ~~shown in Figure 4~~. The amino acid sequence of the predicted protein encoded by the open reading frame is SEQ ID NO. 6 ~~shown in Figure 5~~ and has been termed LLGXL. The first methionine is predicted to be encoded by nucleotide pairs 252-254. The predicted protein is 500 amino acids in length. The first 18 amino acids form a sequence characteristic of a secretory signal peptide (Higgins, D.G., and Sharp, P.M. (1988) Gene 73, 237-244). The propeptide is predicted to have a molecular weight of 56,800 Daltons. Assuming cleavage of the signal peptide at position 18, the unmodified mature protein has a molecular weight of 54,724 Daltons.--

Please delete the paragraph commencing at page 82, line 28.

Please delete the paragraph commencing at page 83, line 11.

Please replace the paragraph commencing on page 85, line 18, with the following re-written paragraph.

-- A commercially prepared filter containing 3 µg each of mRNAs from human tissues (heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas) was



obtained from Clontech (Catalog #7760-1). This filter was probed and processed as described above. After probing with the radiolabeled LLG fragment and autoradiography, the probe was stripped by washing in boiling 0.1X SSC, 0.1% SDS for 2 x 15 min. in a 65°C incubator. The membranes were then probed with a 1.4 kilobase pair DNA fragment encoding human lipoprotein lipase. This fragment was obtained by RT-PCR of the THP-1 RNA (PMA and oxLDL treated) using the 5'LPL and 3'LPL primers depicted in SEQ ID NOS. 20 and 21, respectively, described in Figure 1. and the RT-PCR conditions described above. After autoradiography, the membranes were stripped again and reprobed with a radiolabeled fragment of the human beta actin cDNA to normalize for RNA content. The results of these analyses are shown in Figure 8. The highest levels of LIPG message were detected in placental RNA, with lower levels found in RNAs derived from lung, liver, and kidney tissue. In agreement with previous studies by others (Verhoeven, A.J.M., Jansen, H. (1994) Biochem. Biophys. Acta 1211,121-124), lipoprotein lipase message was found in many tissues, with highest levels found in heart and skeletal muscle tissue. Results of this analysis indicates that the tissue distribution of LIPG expression is very different from that of LPL. The pattern of LIPG expression is also different from that of either hepatic lipase or pancreatic lipase, as reported by others (Wang, C.-S., and Hartsuck, J.A. (1993) Biochem. Biophys. Acta 1166,1-19; Semenkovich, C.F., Chen, S.-W., Wims, M., Luo C.-C., Li, W.-H., and Chan, L. (1989) J. Lipid Res. 30,423-431; Adams, M.D., Kerlavage, A.R., Fields, C., and Venter, C. (1993) Nature Genet. 4,256-265).--

Please replace the paragraph commencing on page 90, line 2, with the following re-written paragraph.

-- Human umbilical vein endothelial cells (HUVEC) and human coronary arterial endothelial cells (HCAEC) were obtained from Clonetics. HUVECs were propagated in a commercially prepared endothelial cell growth medium (EGM, Clonetics) supplemented with 3 mg/ml bovine brain extract (Maciag, T., Cerundolo, J., Ilsley, S., Kelley, P.R., and Forand, R. (1979) Proc. Natl. Acad. Sci. USA 76, 5674-5678), Clonetics), while HCAECs were propagated in EGM supplemented with 3 mg/ml bovine brain extract and 3% fetal bovine serum (5% final concentration). Cells were grown to confluence, then the medium was changed to EGM without bovine brain extract. Cultures were stimulated by adding 100 ng/ml of phorbol myristate (Sigma). After 24 hours incubation, the RNAs were extracted from the cells via the Trizol method described above. Twenty micrograms of total RNA was electrophoresed and transferred to the membrane for analysis. The membranes were probed with LIPG and LPL probes as described above. The results are shown in Figure 9 4. Twenty micrograms of total RNA from THP-1 cells stimulated with PMA was run on the blot for comparison. RNA hybridizing to the LIPG probe was detected in unstimulated and PMA stimulated HUVEC cells. In contrast, detectable levels of LIPG mRNA were only found in HCAEC cultures after stimulation with PMA. In agreement with previous studies of others, no detectable lipoprotein lipase mRNA was detected in any of the endothelial RNAs (Verhoeven, A.J.M., Jansen, H. (1994) Biochem. Biophys. Acta 1211,121-124).--

Pleas replace the paragraph commencing on page 91, line 3, with the following re-written paragraph.

-- Antisera were generated to peptides with sequences corresponding to a region of the predicted protein encoded by the LIPG cDNA open reading frame. This peptide was chosen because of its high predicted antigenicity index (Jameson B.A.,

and Wolf, H. (1988) *Comput. Applic. in the Biosciences* 4,181-186). The sequence of the immunizing peptide was not found in any protein or translated DNA sequence in the Genbank database. Its corresponding position in the LIPG protein is shown in Figure 40 5. The carboxy terminal cysteine of the peptide does not correspond to the residue in the LIPG putative protein, but was introduced to facilitate coupling to the carrier protein. The peptide was synthesized on a Applied Biosystems Model 433A peptide synthesizer. Two milligrams of peptide was coupled to two milligrams of maleimide-activated keyhole limpet hemocyanin following the protocols included in the Inject Activated Immunogen Conjugation Kit (Pierce Chemical). After desalting, one-half of the conjugate was emulsified with an equal volume of Freund's complete adjuvant (Pierce). This emulsification was injected into a New Zealand White rabbit. Four weeks after the initial inoculation, a booster inoculation was made with an emulsification made exactly as described above except Freund's incomplete adjuvant (Pierce) was used. Two weeks after the boost, a test bleed was made and titers of specific antibodies were determined via ELISA using immobilized peptide. A subsequent boost was made one month after the first boost.--

Please replace the paragraph commencing on page 92, line 5, with the following re-written paragraph.

-- HUVEC and HCEAC cells were cultured and stimulated with PMA as described in Example 3C, except that the cells were stimulated with PMA for 48 hours. Samples of conditioned medium (9 ml) were incubated with 500 µl of a 50% slurry of heparin-Sepharose CL-6B in phosphate buffered saline (PBS, 150 mM sodium chloride, 100 mM sodium phosphate, pH 7.2). Heparin-Sepharose was chosen to partially purify and concentrate the LIPG proteins because of the conservation of residues in the LLGXL sequence which have been identified as

critical for the heparin-binding activity of LPL (Ma, Y., Henderson, H.E., Liu, M.-S., Zhang, H., Forsythe, I.J., Clarke-Lewis, I., Hayden, M.R., and Brunzell, J.D. *J. Lipid Res.* 35, 2049-2059; and Fig. 1 6.). After rotation at 4°C for 1 hour, the samples were centrifuged for 5 minutes at 150 x g. The medium was aspirated and the Sepharose was washed with 14 ml PBS. After centrifugation and aspiration, the pelleted heparin-Sepharose was suspended in 200 µl 2x SDS loading buffer (4% SDS, 20% glycerol, 2% β-mercaptoethanol, 0.002% bromphenol blue, and 120 mM Tris pH 6.8). The samples were heated to 95°C for 5 minutes and 40 µl was loaded onto a 10% Tris-Glycine SDS gel. After electrophoresis at 140 V for approximately 90 minutes, the proteins were transferred to nitrocellulose membranes via a Novex electroblotting apparatus (210 V, 1 hour). The membranes were blocked for 30 minutes in blocking buffer (5% nonfat dried milk, 0.1% Tween 20, 150 mM sodium chloride, 25 mM Tris pH 7.2). Antipeptide antisera and normal rabbit serum was diluted 1:5000 in blocking buffer and was incubated with the membranes overnight at 4°C with gentle agitation. The membranes were then washed 4x 15 minutes with TBST (0.1% Tween 20, 150 mM sodium chloride, 25 mM Tris pH 7.2). Goat anti-rabbit peroxidase conjugated antisera (Boehringer Mannheim) was diluted 1:5000 in blocking buffer and incubated with the membrane for 1 hour with agitation. The membranes were washed as above, reacted with Renaissance chemiluminescent reagent (DuPont NEN), and exposed to Kodak XAR-2 film. The results are shown in Figure 6 44. Two species of immunoreactive proteins are present in the samples from unstimulated HUVEC and HCAEC cells. Levels of immunoreactive protein in the unstimulated HCAEC samples are much lower than the corresponding HUVEC sample. Upon stimulation with PMA, three immunoreactive proteins are secreted by the endothelial cell cultures. PMA exposure greatly increased the level of LIPG proteins produced

by the HCAEC cultures. PMA induction of LLG proteins was not as dramatic in the HUVEC cultures.--

Please replace the paragraph commencing on page 93, line 25, with the following re-written paragraph.

-- The cDNAs encoding the LLGN and LLGXL proteins were cloned into the mammalian expression vector pCDNA3 (Invitrogen). This vector allows expression of foreign genes in many mammalian cells through the use of the cytomegalovirus major late promoter. The LLGN 5'RACE product was cloned into the EcoRI site of pCDNA3. The LLGXL cDNA was digested with DraI and SrfI to yield a 1.55 kb cDNA (SEQ ID NO. 5~~see Figure 4.~~). The vector was digested with the restriction enzyme EcoRV and the vector and insert were ligated using T4 DNA ligase and reagents from the Rapid Ligation Kit (Boehringer Mannheim) according to the manufacturers instructions. The ligation products were used to transform competent E. coli . Resultant colonies were screened by restriction analysis and sequencing for the presence and orientation of the insert in the expression vector. --

Please replace the paragraph commencing on page 94, line 13, with the following re-written paragraph.

-- The LIPG expression vectors were introduced into COS-7 cells through the use of Lipofectamine cationic lipid reagent (GIBCO). Twenty-four hours before the transfection, COS-7 cells were plated onto 60 mm tissue culture dishes at a density of  $2 \times 10^5$  cells/plate. The cells were propagated in Dulbecco's modified Eagle's medium (DMEM; GIBCO) supplemented with 10% fetal calf serum, 100 U/ml penicillin, 100

µg/ml streptomycin. One microgram of plasmid DNA was added to 300 µl of Optimem I serum-free medium (Gibco). Ten microliters of Lipofectamine reagent were diluted into 300 µl of Optimem I medium and this was combined with the DNA solution and allowed to sit at room temperature for 30 minutes. The medium was removed from the plates and the cells were rinsed with 2 ml of Optimem medium. The DNA-Lipofectamine solution was added to the plates along with 2.7 ml Optimem medium and the plates were incubated for 5 hours at 37°C. After the incubation, the serum free medium was removed and replaced with DMEM supplemented with 2% FBS and antibiotics. Twelve hours post-transfection, some of the cultures were treated with either 0.25 mM Pefabloc SC (Boehringer Mannheim), a protease inhibitor, or 10 U/ml heparin. Thirty minutes before harvest, the heparin treated samples were treated with an additional 40 U/ml heparin. The medium was removed from the cells 60 hours after transfection. Heparin-Sepharose CL-4B (200 µl of a 50% slurry in PBS pH 7.2) was added to 1 ml of medium and was mixed at 4°C for 1 hour. The Sepharose was pelleted by low speed centrifugation and was washed three times with 1 ml cold PBS. The Sepharose was pelleted and suspended in 100 µl 2x loading buffer. The samples were heated to 95°C for 5 minutes. 40 µl of each sample was loaded onto a 10% SDS-PAGE gel. Electrophoresis and western analysis was performed using the anti-LIPG antiserum as described above. The results are shown in Figure 7-42. Proteins from HCAEC conditioned medium were included for size references. LLGN migrates at approximately 40 kD, corresponding to the lowest band in HCAEC. The medium from COS cells transfected with LLGXL cDNA contains both 68 kD and 40 kD species. When these cells were treated with heparin, the amount of both 68 kD and 40 kD proteins recovered from the medium increased dramatically, indicating either the release of proteoglycan-bound protein from the cell surface or stabilization of the proteins by heparin. When the cells were treated with

the protease inhibitor Pefabloc, the amount of 68 kD protein increased relative to that of the 40 kD species. This suggests that the lower molecular weight protein produced by these cells is a proteolysis product of the larger 68 kD form. The role of the mRNA identified through differential display which encodes a shorter, 40 kD species is not known. There has, however, been a report of an alternately-spliced form of hepatic lipase which apparently is expressed in a tissue-specific manner and would create a truncated protein.--

Please replace the paragraph commencing on page 96, line 10, with the following re-written paragraph.

-- A commercially available lambda cDNA library derived from rabbit lung tissue (Clontech, Cat. #TL1010b) was used to isolate a fragment of the rabbit homolog of the LIPG gene. Five microliters of the stock library were added to 45 µl water and heated to 95°C for 10 minutes. The following were added in a final volume of 100 µl: 200 µM dNTPs, 20 mM Tris-HCl pH 8.4, 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 100 µM each primer DLIP774 and LLGgen2a, and 2.5 U Taq polymerase (GIBCO). The reaction was thermocycled 35 times with the parameters of: 15 seconds at 94°C, 20 seconds at 50°C and 30 seconds at 72°C. Ten microliters of the reaction was analyzed via agarose gel electrophoresis. A product of approximately 300 basepairs was detected. A portion (4 µl) of the reaction mix was used to clone the product via the TA cloning system. The insert of a resulting clone was sequenced (SEQ ID NO: 7 44). An alignment between the deduced rabbit amino acid sequence (SEQ ID NO: 8 42) and the corresponding sequence of the human cDNA is also shown in Figure 9 44. Of the nucleotides not part of either amplification primer, there is an 85.8% identity between the rabbit and human LLG sequences. The predicted protein encoded by this rabbit

cDNA shares 94.6 % identity with that of the human protein, with most of the nucleotide substitutions in the third or "wobble" positions of the codons. Notably, this region spans the "lid" sequence of the predicted LLG proteins and is a variable domain in the lipase gene family. This is evidence that there is a high degree of conservation of this gene between species.--

Please replace the paragraph commencing at page 97, line 15, with the following re-written paragraph.

-- The membranes were hybridized overnight at 65°C with  $2.5 \times 10^6$  cpm/ml of random primed  $^{32}$ P-LLG or  $^{32}$ P-LPL (lipoprotein lipase) probe in a hybridization solution of 6X SSC, 10% dextran sulfate, 5 X Dendardt's solution, 1% SDS, and 5°g/ml salmon sperm DNA. The membranes were washed with 0.1X SSC, 0.5% SDS for ten minutes at room temperature, then sequentially for ten minutes at 40°C, 50°C, and 55°C. Autoradiograms of the blots are shown in Figure 11 16.--

Please replace the paragraph commencing on page 97, line 23, with the following re-written paragraph.

-- Figure 11 16 shows the presence of LLG and LPL genes in all species examined, with the exception that no hybridization was observed with the LLG probe against rat DNA. The exceptional data from rat may represent an artifact caused by generation of abnormally sized restriction fragments containing LLG sequences. Such fragments may be outside of the fractionation range of the agarose gel or may blot inefficiently. The different bands detected by the two probes indicate that LPL and LIPG are separate, evolutionarily conserved genes.--



Please replace the paragraph commencing at page 98, line 20, with the following re-written paragraph.

-- Reactions were performed in duplicate and contained 50 µl PC emulsion and 950 µl medium. Samples were incubated in a shaking water bath for 2-4 hours at 37°C. The reactions were terminated by adding 1 ml 1N HCl, then extracted with 4 ml of 2-propanol:hexane (1:1). The upper 1.8 ml hexane layer was passed through a silica gel column, and the liberated <sup>14</sup>C-free fatty acids contained in the flow-thru fraction were quantitated in a scintillation counter. The results of these assays are shown in Figure 9 14.--

Please replace the paragraph commencing at page 99, line 25, with the following re-written paragraph.

-- Reactions were performed in duplicate in a total volume of 0.2 ml containing 0.1 ml of assay substrate and 0.1 ml of the indicated conditioned media. The reactions were incubated for 90 minutes at 37°C. The reactions were terminated by adding 3.25 ml of methanol-chloroform-heptane 1.41:1.25:1 (v/v/v) followed by 1.05 ml of 0.1M potassium carbonate-borate buffer (pH 10.5). After vigorous mixing for 15 seconds, the samples were centrifuged for 5 minutes at 1000 rpm. A 1.0 ml aliquot of the upper aqueous phase was counted in a scintillation counter. The results of these assays are shown in Figure 10 15.--

Please replace the paragraph commencing at page 101, line 20, with the following re-written paragraph.

--The 1.53 kb *DraI*/*SrfI* restriction fragment encoding LLGXL (~~see Figure 4~~) was cloned into a plasmid vector (pHMG) downstream of the promoter for the ubiquitously expressed 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase gene. Transgenic mice expressing different levels of human LLGXL are generated using standard methods (see, e.g., G.L. Tromp et al. Gene 1565:199-205, 1995). The transgenic mice are used to determine the impact of LLGXL overexpression on lipid profile, vascular pathology, rate of development and severity of atherosclerosis, and other physiological parameters.--

Please replace the paragraph commencing at page 103, line 19, with the following re-written paragraph.

-- The G3PDH primers (SEQ ID NOS. 26 and 27 ~~see Figure 1~~) yielded the expected product of 983 bp in all four vascular biopsy samples. LIPG expression was detected in three of the four samples, with no expression being detected in the carotid artery sample.--

Please replace the paragraph commencing at page 103, line 25, with the following re-written paragraph.

--To perform the experiments discussed in Examples 12 to 16, the following procedure (based on the procedure outlined in Example 1) was used to obtain the cDNA for LIPG. THP-1 cells were plated in the presence of phorbol 12-myristate 13-acetate (PMA, 40 ng/ml; Sigma) for 48 hours. The differentiated THP-1 cells were exposed for 24 hours to either oxLDL (50 µg/ml) or control medium. Total

RNAs were collected and purified using standard procedures. Poly(A)<sup>+</sup> RNA was purified from total RNA using a poly-dT magnetic bead system (Promega). cDNA synthesis and PCR amplification were accomplished using protocols from the Differential Display kit, version 1.0 (Display Systems Biotechnology). The primer pairs that yielded the initial cDNA fragment of EL were downstream primer 7 (5'-TTTTTTTTTTTGA-3') (SEQ ID NO: ~~17~~ 13) and upstream primer 15 (5'-GATCCAATCGC-3') (SEQ ID NO: ~~18~~ 14). The amplification reaction was fractionated on a 6% nondenaturing acrylamide sequencing format gel and an amplification product found only in the reaction containing cDNA from THP-1 cells exposed to oxLDL was identified and excised from the gel. A reamplification using the same primers was performed and the product was excised and subcloned into the pCRII vector using the TA cloning system (Invitrogen). Insert sizes were determined using *EcoRI* digestions of the plasmids, and clones containing inserts of the approximate size of the original PCR product were sequenced using fluorescent dye-terminator reagents (Prism, Applied Biosystems) and an Applied Biosystems 373 DNA sequencer. We extended the cDNA sequence of the original, gel-excised cDNA using the 5'-RACE system (GIBCO). RNA (1 µg) from the THP-1 cells used initially in the differential display reactions was used in the 5'-RACE procedure using a gene-specific primer (5'-TAGGACATGCACAGTGTAATCTG-3') (SEQ ID NO: 19) for first strand cDNA synthesis. We performed PCR amplification of the cDNA using an anchor primer and gene-specific primer 2 (5'-GATTGTGCTGGCCACTTCTC-3') (SEQ ID NO: ~~20~~ 16). This reaction (1 µl) was used in a nested re-amplification using the universal amplification primer (5'-CUACUACUACUAGGCCACGCGTCGACTAGTAC-3') (SEQ ID NO: ~~22~~ 18) and the gene-specific primer 3 (5'-GACACTCCAGGGACTGAAG-3') (SEQ ID NO: ~~24~~ 17) to increase levels of specific product for subsequent isolation. The reaction

products were cloned into the pCRII vector from the TA cloning kit and the sequence determined. A human placental cDNA library (oligo dT and random primed) was obtained from Clontech and probed with the 5'-RACE reaction PCR product. The DNA from hybridizing clones was purified using LambdaSorb reagent (Promega). Inserts were excised from the phage DNA by digestion with *EcoRI*, subcloned into the *EcoRI* site of the Bluescript II SK plasmid vector (Stratagene), and sequenced.--

Please replace the paragraph commencing at page 105, line 17, with the following re-written paragraph.

--A 17-residue peptide (GPEGRLEDKLHKPKATC) (SEQ ID NO: 16 12) was synthesized corresponding to residues 8-23 of the secreted LIPG gene product on a Model 433A peptide synthesizer (Applied Biosystems). Peptide (2 mg) was coupled to maleimide-activated keyhole limpet haemocyanin (2 mg) following the protocols included in the Inject Activated Immunogen Conjugation kit (Pierce Chemical). After desalting, one-half of the conjugate was emulsified with an equal volume of Freund's complete adjuvant (Pierce) and injected into a New Zealand White rabbit. Four weeks after the initial inoculation, a booster inoculation was administered with an emulsification made exactly as described above except for the use of Freund's incomplete adjuvant (Pierce). Two weeks after the boost, the titres of specific antibodies were determined in a test bleed via ELISA using immobilized peptide.--

Please replace the paragraph commencing at page 106, line 6, with the following re-written paragraph.

--HUVECs were propagated in a commercially prepared endothelial cell growth medium (EGM, Clonetics) supplemented with bovine brain extract (3 mg/ml; Clonetics), whereas HCAECs were propagated in EGM with bovine grain extract (3 mg/ml) and 5% fetal bovine serum. Cultures were stimulated by addition of PMA (100 ng/ml). After 24 hours incubation, RNA was extracted from the cells via the Trizol method, electrophoresed on a 1% agarose-formaldehyde gel, transferred to Nytran membrane on a Turboblotter apparatus (Schleicher and Schuell) and crosslinked to the membrane using a Stratalinker ultraviolet crosslinker (Stratagene). The 5'-RACE reaction PCR product was radiolabelled using the random priming technique. The radiolabelled probe ( $1-2 \times 10^6$  cpm/ml) was denatured by heating to 95 °C for 10 minutes and quick-chilled on ice before adding to the filter in QuikHyb. Hybridization was allowed to proceed for 3 hours at 65 °C. Filters were exposed to Kodak XAR-2 film with intensifying screens at -80 °C. We incubated HUVEC- and HCEAC-conditioned medium with heparin-Sepharose CL-6B at 4 °C for 1 hour. After centrifugation, the pelleted heparin-Sepharose was suspended in SDS loading buffer, heated to 95 °C for 5 minutes and loaded onto a 10% Tris-Glycine SDS gel (NOVEX). After electrophoresis at 140 V for 90 minutes, the proteins were transferred to nitrocellulose membranes and detected with rabbit anti-LIPG peptide antisera (1:5,000), with goat anti-rabbit peroxidase conjugated antisera (1:5,000; Boehringer) as the secondary antibody. The membranes were reacted with Renaissance chemiluminescent reagent (DuPont NEN) and exposed to Kodak XAR-2 film. A commercially prepared filter containing poly(A)<sup>+</sup> RNAs (3 µg each) from human heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas (Clontech) was hybridized with a radiolabelled fragment and processed as described above. Following autoradiography, the blot was stripped

by washing in boiling 0.1xSSC, 0.1% SDS for 2x15 minutes at 65 °C and then probed as described above with a 1.4-kb cDNA fragment encoding human LPL. This fragment was obtained by RT-PCR of the THP-1 RNA (PMA and oxLDL treated) using the 5' LPL and 3' LPL primers 5'-ACCACCATGGAGAGCAAAGCCCTG-3' (SEQ ID NO: 24 20) and 5'-CCAGTTTCAGCCTGACTTCTTATTC-3' (SEQ ID NO: ~~25~~ 21), respectively. After exposure to film, the membranes were stripped again and reprobed with a radiolabelled fragment of human  $\beta$  actin cDNA to normalize to RNA content.--

Please replace the paragraph commencing at page 107, line 21, with the following re-written paragraph.

--Human umbilical vein endothelial cells (HUVEC) were negative for LPL mRNA expression as expected, but were found to constitutively express a high level of mRNA for the LIPG gene (Figure 4 9).--

Please replace the paragraph commencing at page 107, line 25, with the following re-written paragraph.

--Human coronary artery endothelial cells (HCAEC) were also found to express the mRNA which was further upregulated on treatment of these cells with phorbol ester (Figure 4 9).--

Please replace the paragraph commencing at page 108, line 1, with the following re-written paragraph.

--Conditional medium from stimulated HUVEC and HCAEC contained immunoreactive proteins of approximately 68 kD and 40 kD, as well as a less prominent band of 55 kD (Figure 6 ~~44~~).--

Please replace the paragraph commencing at page 108, line 4, with the following re-written paragraph.

--To determine the tissue sites of LIPG production *in vivo*, a multiple human tissue northern blot analysis with probes for both LIPG and LPL was performed. Abundant levels of LIPG mRNA were found in lung, liver and kidney (Figure 3 ~~8~~) tissues, which showed low levels of LPL expression. LIPG was also expressed at high levels in the placenta (Figure 3 ~~8~~), suggesting the potential for a role in development.--

Please replace the paragraph commencing on page 111, line 14, with the following re-written paragraph.

-- Intravenous injection of AdhEL into wild-type C57BL/6 mice resulted in expression in the liver (Figure 12 ~~47~~) and reduction of plasma levels of HDL cholesterol that remained significantly lower than control virus-injected mice through at least 41 days post-injection (Figure 13 ~~48~~). Lipoproteins were separated by FPLC gel filtration, demonstrating that HDL was undetectable 14 days after adenovirus injection (Figure 14 ~~49~~). Injection of recombinant LIPG adenovirus into human apoA-I transgenic mice (which have much higher levels of HDL cholesterol and apoA-I) reduced both HDL cholesterol (Figure 15 ~~20~~) and apoA-I (Figure 16 ~~24~~) levels. To determine the relative effects of LIPG expression on HDL compared with the apoB-containing lipoproteins VLDL and LDL, we injected a lower dose of the LIPG

adenovirus into chow-fed LDL receptor-deficient mice, which have approximately 70% of cholesterol in VLDL/LDL and approximately 30% in HDL. As before, expression of LIPG reduced HDL cholesterol levels (Figure 18 ~~23~~). Although LIPG expression reduced VLDL/LDL cholesterol levels in the same mice (Figure 19 ~~24~~), the effect was proportionately less. Overexpression of LIPG reduced VLDL/LDL cholesterol, therefore a role of LIPG in the modulation of apoB-containing lipoproteins cannot be excluded.--

Please delete all previous Sequence Listings and replace them with the enclosed revised Sequence Listing.



In the Drawings

Please replace the Figures with the attached Figures 1 to 18. Former Figures 1 to 5 have been deleted and former Figures 6 to 23 have been renumbered as Figures 1 to 18.